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Description

This invention relates to a method of cultivating animal and plant cells. More specifically, it relates to a method of cultivating animal and plant cells in a cultivation system into which oxygen is easily and efficiently supplied, whereby their growth is markedly increased.

Cultivation of animal cells, especially in large quantities, is essential to the production of antiviral agents such as viral vaccines and interferon or biologically active chemicals such as hormones. In particular, the production of monoclonal antibodies having the ability to bind to a specified protein as a target relies on the cultivation of a large quantity of a hybridoma obtained by fusing antibody-producing cells with myeloma cells, and the solution of problems associated with this technique is an industrially important subject.

Heretofore, cell culture has been carried out on a laboratory scale by using a Petri dish, a test tube, a cultivation bottle, etc. Generally, the cell culture is classified into anchorage dependent cell culture and suspension culture depending upon the cells to be cultivated. The suspension culture would be an industrially advantageous method since it has a possibility of cultivating cells at a high density.

Cell cultures usually demand supply of oxygen (O_2), and for this purpose, the concentration of oxygen in the suspension is maintained constant by, for example, supplying an oxygen-containing gas from the gaseous phase above the liquid surface of the suspension and dissolving it in the suspension, or by blowing an oxygen-containing gas into the suspension. The supply of oxygen by these methods does not particularly give rise to any problem in the cultivation of cells on a small scale.

But where cell culture is desired to be effected on an industrial scale, above all at a high cell density, all of the above methods of oxygen supply are unsuitable. In the case of supplying oxygen from the free liquid surface of the suspension, even when the amount of the suspension increases, the area of the liquid surface cannot be increased correspondingly. Thus, on an industrial scale, it is almost impossible to avoid the insufficiency of oxygen supply.

When the oxygen-containing gas is blown into the suspension, the liquid surface rises owing to bubbling, and it is sometimes even difficult to continue the operation. Furthermore, this method is difficult to apply to cells which might die or decrease in proliferating activity upon contact with bubbles, or to cells which undergo a separation phenomenon by the action of bubbles (a certain kind of plant cells, for example).

Recently, a different method was proposed in Japanese Patent Publication No. 4235/1982. This patent document discloses a method of cultivating cells which comprises adhering cells to the surface of a semipermeable containing a quaternary ammonium salt having a water content of 20 to 90 %, and passing a cultivation fluid over at least the opposite side to the cell-adhering surface of the membrane. This method is characterized by the fact that nutrients and oxygen are supplied to the cells through the semipermeable membrane without direct contact of the cells with the cultivation fluid.

The cultivation of plant cells has the same problem as the cultivation of animal cells. It is known that the cultivation of plant cells can give the same substances as primary or seconday metabolites obtained from the parent plants, such as enzymes, terpenoids, flavonoids, steroids, alkaloids, quinones and phenols, and useful substances in the fields of medicines, foods, cosmetics and fine chemicals. It is very important therefore to solve the same problem of the cultivation of plant cells as in the case of the cultivation of animal cells.

Science, volume 219, pages 1448-1449, March 1983 discloses a method in which a fluorocarbon emulsified and stabilized with polylysine is used as a microcarrier, and anchorage-dependent cells are cultivated on the microcarrier.

U. S. Patent No. 3,850,753 discloses a method of cultivating an aerobic microorganism in the presence of a water-immisicible inert liquid fluorocarbon under aeration, agitation and/or shaking.

It is an object of this invention to provide a novel method of suspension cultivation of animal and plant cells.

Another object of this invention is to provide an industrially advantageous cultivation method whereby an amount of oxygen necessary and sufficient for the proliferation of animal and plant cells can be supplied to a suspension cultivation system even at a considerably high cell density, for example, at least 4×10^6 cells/ml for animal cells, by the presence of a liquid fluorocarbon showing a specific range of surface area in the suspension cultivation system, and the liquid fluorocarbon which has supplied oxygen can be easily separated and recovered from the animal or plant cells.

Still another object of this invention is to provide a novel cultivation method whereby an amount of oxygen necessary and sufficient for proliferation can be supplied very smoothly and easily to animal or plant cells substantially without any deleterious effect on the cells.

Yet another object of this invention is to provide a high density cultivation method in which oxygen can

be supplied smoothly to a cultivation fluid having animal or plant cells suspended therein whereby the cell density can be increased to at least 10 times, for example, in comparison with a conventional method.

A further object of this invention is to provide an industrially advantageous cultivation method in which oxygen can be supplied to a cultivation fluid having animal or plant cells suspended therein without vigorously bubbling the cultivation fluid whereby deactivation of a useful metabolite accumulated in the cultivation fluid can be inhibited.

A still further object of this invention is to provide a method useful for industrial cultivation, in which a fluorocarbon which has supplied molecular oxygen is withdrawn from the cultivation system, and after blowing molecular oxygen into it, recycled to the cultivation system.

An additional object of this invention is to provide a method of cultivating animal or plant cells susceptible to damage by agitation or the like in which without performing an external operation of positively agitating the cultivation system, the cultivation fluid moves incident to the falling of a fluorocarbon through the cultivation system and a gentle but effective agitating action occurs in the cultivation system whereby the animal or plant cells can be cultivated without damage by the agitation.

Other objects of this invention along with its advantages will become apparent from the following description.

In accordance with this invention, the above objects and advantages of this invention are achieved by a method of cultivating animal or plant cells, which comprises contacting a cultivation liquor having animal or plant cells suspended therein with a liquid fluorocarbon having molecular oxygen dissolved therein while one of the cultivation liquor and the liquid fluorocarbon is present in a continuous phase of the other such that 1 ml of it shows a surface area of about 2 to about 600 cm^2.

According to the method of this invention, the cultivation liquor having animal or plant cells suspended therein and the liquid fluorocarbon having molecular-oxygen dissolved therein are directly contacted with each other whereby through the contacting surfaces of the two phases, oxygen from the liquid fluorocarbon phase dissolves in the cultivation liquor and an amount of oxygen necessary and sufficient for cultivation of the animal cells can be supplied.

Investigations of the present inventors have shown that even when the aforesaid direct contact of the liquid fluorocarbon with the cultivation liquor results in direct contact between the cells and the fluorocarbon, the cells are stable both chemically and physically and proliferate without any adverse effects on their growth, and that cultivation of the cells at a high density which requires a large amount of oxygen can be achieved without any troubles even when the fluorocarbon is supplied in a large quantity.

In the method of this invention, either one of the cultivation liquor and the liquid fluorocarbon may form a continuous phase. The other forms a phase separated by the continuous phase. The cultivation liquor or the liquid fluorocarbon as the separated phase should be present in the other continuous phase such that 1 ml of the separated phase shows a surface area of about 2 to about 600 cm^2. The surface area shown by 1 ml of the separated phase is preferably about 6 to about 300 cm^2, more preferably 10 to 200 cm^2. The surface area of the separated phase does not include that of an interface between the continuous phase and a precipitated phase (when the separated phase is the fluorocarbon) formed after it has gone past the continuous phase or a floating phase (when the separated phase is the cultivation liquor). The formation of the precipitated phase and the floating phase will be described hereinafter.

The liquid fluorocarbon used in the method of this invention has an excellent ability to dissolve molecular oxygen in regard to the amount and the speed in and at which it dissolves molecular oxygen. The liquid fluorocarbon is not miscible with the cultivation liquor, and is substantially nontoxic to animal or plant cells. Preferably, the liquid fluorocarbon used in the method of this invention is a liquid perfluorocarbon. Examples of the liquid fluorocarbon include perfluoroalkanes having 6 to 20 carbon atoms, perfluorocycloalklanes having 5 to 14 carbon atoms which may be substituted by perfluoroalkyl groups having 1 to 5 carbon atoms, perfluorotetrahydrofurans substituted by perfluoroalkyl groups having 1 to 5 carbon atoms, perfluorotetrahydropyrans substituted by perfluoroalkyl groups having 1 to 5 carbon atoms, perfluoroadamantanes which may be substituted by perfluoroalkyl groups having 1 to 5 carbon atoms, and tertiary amino group-substituted products of the above fluorocarbons.

Specific examples of the perfluoroalkanes having 6 to 20 carbon atoms are linear or branched perfluoro-n-heptane, perfluorooctane and perfluorononane.

Specific examples of the perfluorocycloalkanes having 5 to 14 carbon atoms which may be substituted by perfluoroalkyl groups having 1 to 5 carbon atoms are perfluoro-1-trimethyldecalin and perfluorodecalin.

Specific examples of the perfluorofurans or perfluorotetrahydrofurans substituted by perfluoroalkyl groups having 1 to 7 carbon atoms are perfluoro-2-butylperfluorofuran, perfluoro-2-butyltetrahydrofuran, perfluoropentyl perfluorofuran, perfluoropentyl tetrahydrofuran, perfluorohexyl perfluorofuran, perfluorohexyl tetrahydrofuran, perfluoroheptyl perfluorofuran, and perfluoroheptyl tetrahydrofuran.

Specific examples of the perfluorfurans or tetrahydropyrans substituted by perfluoroalkyl groups having 1 to 6 carbon atoms include perfluorobutyl perfluoropyran, perfluorobutyl tetrahydropyran, perfluoropentyl perfluoropyran, perfluoropentyl tetrahydropyran, perfluorohexyl perfluoropyran, and perfluorohexyl tetrahydropyran.

Specific examples of the perfluoroadamantanes which may be substituted by perfluoroalkyl groups having 1 to 5 carbon atoms are perfluoroadamantane, perfluoromethylperfluoroadamantane, perfluorodimethylperfluoroadamantane, perfluoromethylperfluoroethylperfluoroadamantane and perfluorodimethylperfluoroadamantane.

Examples of the tertiary amino group substituted products of these fluorocarbons are perfluorotributylamine-substituted products and perfluoro-N-methylmorpholine substituted products of these fluorocarbon.

These liquid fluorocarbons may be used singly or in combination.

As stated hereinabove, the method of this invention is carried out while one of the cultivation liquor and the liquid fluorocarbon is used as a continuous phase and the other, as a separated phase. In other words, the method of this invention includes a first embodiment in which the cultivation liquor is the continuous phase and the fluorocarbon is the separated phase, and a second embodiment in which the fluorocarbon is the continuous phase and the cultivation liquor is the separated phase. In any of these embodiments, the separated phase is present in the continous phase while it shows the surface area specified hereinabove. In such a state, the separated phase may be in the form of, for example, a liquid droplet, a liquid column, or a liquid film. When the separated phase is in the form of a liquid phase, it is preferred that 1 ml of it forms a liquid droplet showing a surface area of about 3 to about 600 cm^2, especially 15 to 100 cm^2. When the separated phase is in the form of a liquid column, it is preferred that 1 ml of it forms a liquid column showing a surface area of about 4 to about 100 cm^2. When the separated phase is in the form of a liquid film, it is preferred that 1 ml of it forms a liquid film showing a surface area of about 2 to about 50 cm^2.

Generally, the liquid fluorocarbon shows a specific gravity about 1.6 to about 2 times that of water. Accordingly, in the practice of the method of this invention, it is advantageous to utilize this high specific gravity of the liquid fluorocarbon.

For example, in the first embodiment in which the cultivation liquor forms a continuous phase, the liquid fluorocarbon having molecular oxygen dissolved therein may be fed into the continuous phase from above the continuous phase whereby the liquid fluorocarbon, in the form of, for example, a liquid droplet, column or film, falls by gravity through the continuous phase.

In the second embodiment in which the liquid fluorocarbon forms a continuous phase, the cultivation liquor may be fed into the continuous phase from below the continuous phase whereby the cultivation liquor, in the form of a liquid droplet, column or film, can rise by the difference in specific gravity through the liquid fluorocarbon

In the first embodiment, the liquid fluorocarbon which has fallen by gravity through the cultivation liquor supplies molecular oxygen to the cultivation liquor and forms a precipitated phase upon arrival at the bottom of the cultivation tank. The precipitated phase can be separated from the cultivation liquor by a suitable operation. In the second embodiment, the cultivation liquor which rises through the liquid fluorocarbon by the difference in gravity receives the supply of molecular oxygen from the liquid fluorocarbon and forms a floating phase on the fluorocarbon continuous phase upon arrival at the top of the cultivation tank.

In any of these embodiments, as the separated phase moves either downwardly or upwardly through the continuous phase as a liquid drop, column or film, an agitation action occurs in the continuous phase. This agitating action is gentle but very efficient, and for this reason, molecular oxygen can be smoothly supplied from the liquid fluorocarbon to the cultivation liquor without applying a positive agitating operation externally to the cultivation system.

The precipitated phase in the first embodiment and the floating phase in the second embodiment can be recycled to the cultivation system. For example, the cultivation method involving recycling of the precipitated phase can be advantageously carried out by a combination of the following four steps.

(1) Feeding a liquid fluorocarbon having molecular oxygen dissolved therein from above into a cultivation tank containing a continuous phase of a cultivation liquor having animal or plant cells suspended therein to thereby contact them with each other while 1 ml of the liquid fluorocarbon shows a surface area of about 2 to about 600 cm^2;

(2) withdrawing a heavy phase (precipitated phase) composed substantially of the fluorocarbon from the bottom of the cultivation tank;

(3) dissolving molecular oxygen in the fluorocarbon in the heavy phase, and

(4) thereafter using the liquid fluorocarbon having molecular oxygen dissolved therein in step (1).

On the other hand, according to the method involving recycling the floating phase in the second embodiment, a light phase (floating phase) composed of the cultivation liquor is taken out from the top of the cultivation tank, and recycled and fed from below into the fluorocarbon phase forming a continuous phase. Recycling is carried out until the desired cultivation proceeds, and in the meanwhile, moleuclar oxygen is supplied continuously or stepwise to the fluorocarbon phase.

Furthermore, the method of this invention, especially in the first embodiment, can be conveniently carried out by a perfusion method which comprises separating and withdrawing the cultivation liquor from the cultivation system composed substantially of the liquid fluorocarbon, the cultivation liquor and the animal or plant cells, continuously or stepwise, and supplying a fresh liquid medium in an amount corresponding to the withdrawn cultivation liquor to the cultivation system continuously or stepwise.

The fresh liquid medium contains nutrients, salts, etc. required for cultivation. When animal cells are to be cultivated, the fresh liquid medium is formed by adding components normally used in cell cultures, such as various inorganic salts, vitamins, coenzymes, glucose, amino acids and antibiotics to an aqueous medium composed substantially of water. Serum may be added to the culture medium. It is also possible to use a so-called serum-free medium for cultivation. When plant cells are to be cultivated, the fresh liquid medium is formed by adding components normally used in plant cell cultures, such as various inorganic salts, vitamins, inositol, sucrose, and plant hormones (such as auxin and cytokinin). It is also possible to add amino acids, coconut milk, casein hydrolyzate, yeast extracts and glucose to the cultivation liquor. Usually, the cultivation liquor is used in a pH range of 4.0 to 6.5.

The cultivation liquor may be separated from the cultivation system by, for example, using a microfilter, a semipermeable membrane, etc. Since the cultivation liquor withdrawn from the cultivation system contains wastes and metabolites of the cells, etc. and other growth inhibiting substances, its withdrawing from the cultivation tank is desirable for efficient proliferation of cells. It is particularly advantageous to grow the cells at a high density.

The cultivation method of this invention can be applied both to animal and plant cells.

Examples of the animal cells to which the method of this invention can be applied include cells capable of producing useful substances, such as BALL-1 cells, TALL-1 cells, NALL-1 cells (I. Miyoshi, Nature, 267, 843, 1977), Namalwa cells (Journal of Clinical Microbiology, 1, 116, 1975), M-7002 cells, B-7101 cells (Journal of Immunology, 113, 1334, 1974), Flow 7000 cells (Flow Company, U. S. A.), JBL cells, EBV-SA cells, EBV-WA cells, and FBV-HO cells ("Tissue Culture", 6, 527, 1980) which are lymphotoxin-producing cells; JAX cells such as Jurket cells or Jurket-FHCRC cells, JP111 (ATCC CRL 8120) which are interleukin 2-producing cells; Namalwa cells which are IFN-producing cells; and hybridomas, transformed cells, cancer cells, and genetically manipulated cells.

Examples of the hybridomas are mouse-mouse hybridoma, mouse-human hybridoma, human-human hybridoma and (mouse-human)-human hybridoma. Parent strains of these hybridomas, are for example, P3-NS1-1-AG4-1, P3-X63-Ag8, P3-X63-Ag8-U1, MPC11-45.6.TG1.7, SP2/0-Ag14X63-Ag8-6.5.3, 210RCY.Ag1.2.3, SKO-007GM15006 TG-A12, and cells transfected with viruses. Examples of cells to be fused with these parent strains are B cells, T cells and macrophage.

Examples of the transformed cells are cells transformed with EB virus and SV40 transformed human fetal lungs.

Examples of the cancer cells are myeloma, hepatoma, carcinosarcoma, osteosarcoma, melanoma, colon adenocarcinoma and medulloblastoma.

The genetically manipulated cells include, for example, those in which the host cells are mouse L cells, NIH/3T3, CHO (ovary cells of Chinese hamster), ovary cells of armyworms, mouse myeloma, and C127 (epithelial cells derived from mouse breast cancer).

Those anchorage-independent animal cells can be directly suspended in the liquid cultivation medium without carrying them on a carrier. Or they may be immobilized with a gel before suspending them in the liquid cultivation medium. On the other hand, anchorage-dependent animal cells can be suspended in the cultivation liquor while carrying them on a carrier. Such anchorage-dependent cells are, for example, vero cells, and human diploid foreskin fibroblasts.

According to the method of this invention, animal cell densities of at least 4×10^5 cells/ml in the cultivation liquor may be preferably employed in the cultivation of these animal cells.

The plant cells to which the method of this invention can be applied include, for example, those derived from higher plants or phanerogamous plants. These cells include those modified artificially or by gene manipulation. Modification may be performed between cells of the same kind having different cell properties with respect to, for example, the content of a useful substance and the speed of cell proliferation, or between cells of taxonomically different plants in terms of species, genus and family.

Specific examples include cells of Nicotiana tabacum, Nicotiana rustica, Atropa belladonna, Datura

stramonium, Datura innoxia, Datura meteloides, Datura tatula, Datura metel, Hyoscyamus niger, Duboisia myoporoides, Scopolia, japonica, Scopolia poruiflora, Peganum harmala, Conium maculatusm, Catharanthus roseus, Rauwolfia serpentian, Digitalis purpurea, Digitalis lanata, Papauer somniferum, Papaver bracteatum, Vincaminr, Coptis japonica, Phellodendron amurense, Phaseolus vulgaris, Camptotheca acuminate, Cephalotaxus harringtonia, Triptergium wilfordii, Ruta graveolens, Phytolacca americana, Agrostemma githago, Beta vulgaris, Cucurma longa, Chenopodium centrorubrum, Derris elliptica, Chrysanthmum cinedrariaefolium, Stephania cepharantha, Isodun japonicus, Dioscorea deltoidea, Crocus sativus, Dioscorea japonica, Dioscorea emposita, Dioscorea tokoro, Sophora angustifolia, Stevia rebaudiana, Yuicca glauca, Achyranthes japonica, Panax ginseng, Lithospermum erythrorhizon, Rheum palmatus, Carthamus tinctorius, Bupleurum falcatum, Cassia angustifolia, Salvia miltiovhiza, Cassia obutsifolia, Morinde citrifolia, Mucuna pruriens, Erythroxylon coca, Colchicum autumnale, Ailanthus altissima, Brucea antidysenterica, Maytenus buchanani, Epimedium grandiflorum, Putterlickia vercosa, Cephaelis ipecacuanba, Glycyrrhiza glabra, Gynostemma pentaphyllum, Heliotroplum indicum, Podophyllum emodi, Jaxus brevifolia, Rose damascona, Rose centifolia, Jasminum officinale, Jasminum grandiflorum, Matricaria chamomilla, Lavendula officinalis, Angelica archangelica, Salvia offcinalis, Mentha arvensis, Mentha piperita, Mentha viridis, Pelargonium denticulatum, Hydrangea serrata, Cochlearia oblongifolio, Capsicum annuum, Achras sapota, Wasabia japonica, Euphorbia tirucalli, Oryza sativa, Triticum vulgare, Hordeum vulgare, Hordeum clistichum, Zea mays, Sorghum nervosum, Glycine max, Phaseolus vulgaris, Pisum sativum, Ipomoea batatas, Dioscorea batatas, Allium cepa, Allium fislulosum, Allium satwum, Solanum tuberosum, Manihot utilissima, Raphanus sativus, Daucus carota, Coloacsia plantaginea, Brassica napus, Helianthus annuus, Spinacia oleracea, Asparagus officinalis, Brassica oleracea, Brassica pekinensis, Apium graveolens, Retroselinum sativum, Lactuca sativa, Brassica rapa, Fragaria chiloensis, Vicia faba, Hycoipersicum esculentum, Solanum melonyena, Citrullus vulgaris, Cucumis sativus, Cucumis melo, Ananas comosus, Cucurbita maxima, Cucurbita pepo, Trifolium pratense, Trifolium repens, Medicago sativa, Dactylis glomerata, Phleum pratens, Musa sapientum, Cocos nucifera, Diospyros kaki, Prunus persica, Malus pumila, Prunus avium, Prunus carasus, Pyrus serotina, Pyrus communis, Prunus communis, Vitis vinifera, Citrus lemon, Citrus unshiu, Citrus sinensis, Olea europaea, Vaccinium pennsylvanicum, Vaccinium canadense, Gossypium hirsutum, Gossypium barbadense, Sossypiuum arboreum, Cammellia sinensis, Cofea arabica, Humulus lupulus, Saccharum officinarum, Elaeis guineensis, Chrysanthemum morifolium, Dianthus caryophyllus, Rosa chinensis, Lillium longiflorum, Lilium aurantum, Tulipa generiana, Gerbera jamesonii and Narcissus tazetta.

These cells may be fused cells obtained between cells or cultivated cells, or between cultivated cells and cells directly obtained from plant tissues, or between cells obtained directly from plant tissues.

The following examples illustrate the present invention more specifically.

In these examples, the surface area of a separated phase in a continuous phase in the cultivation system was measured by the following method.

The size of the separated phase is examined from the side of the cultivation tank (preferably when the separated phase begins to form), and the surface area is calculated from the examined size.

When the separated layer is in the form of liquid droplets, the cultivation system is photographed from the side of the cultivation tank. The sizes of all of the liquid droplets (separated phase) having a clear contour in the photograph are calculated, and the average surface area of the separated phase is calculated from the calculated sizes.

When the separated phase is in the form of a liquid column or film, the cultivation system is photographed from the side of the cultivation tank at various angles so that its sectional area taken in a direction perpendicular to the vertical direction can be calculated as accurately as possible. The sectional areas are calculated from the resulting photographs, and the average surface area of the separated phase is calculated from the calculated values.

EXAMPLE 1

A device shown in Figure 1 of the accompanying drawings was used. A glass fermentor 1 (having a diameter of 80 mm and a height of 140 mm) including a cylindrical rotating filter having a height of 35 mm and an outside diameter of 60 mm (impermeable to cells but permeable to liquid components; G-5 glass fiber in the present example) was sterilized in on autoclave. The fermentor was then charged with 225 ml of RPMI 1640 medium containing 10% fetal bovine serum, and mouse-mouse hybridoma 4C10B6 cells (parent strain: P3U1) were seeded at a density of 1.5×10^5 cells/ml (these cells produce IgG2b antibody). Air containing 5% CO_2 was passed through the top portion of the fermentor, and the cells were cultivated at 37 °C while rotating the rotating filter at 150 rpm.

On the third day, the cell density was measured. Living cells were found to exist at a density of 9.2 x

10^5 cells/ml, and no dead cells were observed. The antibody concentration was 45 micrograms/ml.

On the third day and thereafter, a fresh medium of the above composition was continuously fed at a rate of 450 ml/day from line 14 in Figure 1. Simultaneously, the cultivation liquor was continuously separated from the cells and taken out of the system through line 14 via filter 15 by means of a pump fitted to the end of the line 13 so that the volume of cultivation liquor in the fermentor became constant at 225 ml. On the 4th day, the concentration of dissolved oxygen in the fermentor dropped below 3 ppm. Therefore, the supplying of air containing 5% CO_2 was stopped, and from line 7, Fluorinert FC-40 (a trade name for a fluorocarbon produced by 3M Company) having oxygen dissolved therein was added dropwise from the top of the fermentor so that the amount of dissolved oxygen in it became 3 ppm. The average surface area of the fluorocarbon at this time was 11 cm^2/ml.

The fluorocarbon settled to the bottom of the fermentor, but was taken out of the system through line 6 so that the interface between it and the cultivation liquor was maintained at a constant level. On the 6th day and thereafter, the rate of feeding the fresh medium from line 1 was changed to 675 ml/day.

The operability of the above high-density continuous cultivation by the perfusion method was good. The cell densities and the antibody concentrations in the cultivation liquor were as follows.

Table 1

Time	Cell density (cells/ml)		Concentration of the antibody (micrograms/ml)
	Living cells	Dead cells	
1st day	1.5×10^5	0	-
3rd day	9.2×10^5	0	45
4th day	2.0×10^6	0	-
5th day	4.0×10^6	2.4×10^5	68
6th day	6.2×10^6	3.4×10^5	97
7th day	8.3×10^6	2.7×10^5	97
8th day	1.1×10^7	2.9×10^5	130

COMPARATIVE EXAMPLE 1

A device shown in Figure 2 was used. A gas blowing tube (a glass tube fitted at its tip with a G-3 glass filter) was fitted to a fermentor of the same size as used in Example 1, and the device was sterilized in an autoclave. Thereafter, 225 ml of RPMI 1640 medium containing 10% fetal bovine serum was charged into the fermentor, and mouse-mouse hybridoma 4C10B6 strain (parent strain: P3U1) was seeded in the medium at a cell density of 1.5×10^5 cells/ml. This cell was capable of producing IgG_{2b} antibody. Air containing 5% of CO_2 was passed through the upper portion of the fermentor, and the cultivation was carried out at 37 °C while the rotating filter was rotated at 150 rpm. The cell density measured on the third day was 1.1×10^6 cells/ml, and no dead cell was observed. The concentration of the antibody produced was 48 micrograms/ml.

On the third day, continuous feeding of a fresh medium of the above composition from line 14 in Figure 2 was started at a rate of 450 ml/day. At the same time, the cultivation liquor was continuously separated from the cells and taken out of the system through line 13 via a filter 15 by means of a pump fitted to the end of line 13 so that the volume of the cultivation liquor in the fermentor was maintained constant at 225

ml. On the 4th day, the concentration of dissolved oxygen in the fermentor fell below 3 ppm. Therefore, air containing 5% CO_2 was blown into the fermentor from gas-blowing line 7 so that the concentration of dissolved oxygen became 3 ppm. As soon as the air blowing was started, a bubble layer having a thickness of about 10 mm was formed in the upper portion of the cultivation liquor. The cell densities were measured. The density of living cells was 1.7×10^6 cells/ml, and the density of dead cells was 3.0×10^5 cells/ml. On the fifth day, the height of the bubble layer became about 30 mm. The density of living cells was 3.8×10^6 cells/ml, and the density of dead cells was 1.2×10^6 cells/ml. The concentration of the antibody was 58 micrograms/ml. On the 6th day, the bubble layer became high, and flowed out into gas discharging line 6 to make the continuous operation impossible. Therefore, the experiment was stopped. At this time, the density of living cells was 5.1×10^5 cells/ml, and the density of dead cells was 2.1×10^6 cells/ml. The concentration of the antibody was 80 micrograms/ml.

EXAMPLE 2

A 4-liter fermentation spinner flask made by Shibata Haio Company was charged with 4 liters of RPMI 1640 medium containing 10% fetal bovine serum. Human T-cell line MLT 2.2 was seeded at a cell density of 1×10^5 cells/ml, and the cultivation was started at 37 °C at a speed of 60 rpm. Air containing 5% CO_2 was passed through the upper portion of the fermentor, and beginning on the second day after the start of the fermentation, a fluorocarbon (Fluorinert FC-40) saturated in advance with oxygen was added dropwise so that the concentration of dissolved oxygen in the fermentor became 3 ppm. The cultivation was continued. The average surface area of the fluorocarbon at this time was 10 cm^2/ml. On the second day, the density of living cells was 3.2×10^5 cells/ml, and on the 5th day, it was 9.8×10^5 cells/ml.

COMPARATIVE EXAMPLE 2

Cultivation was started under the same conditions as in Example 2 except that the feeding of the fluorocarbon to the cultivation system was not effected. In this experiment, the density of living cells on the 2nd day was 2×10^5 cells/ml, and on the 5th day, it was 1.8×10^5 cells/ml.

EXAMPLES 3-7 AND COMPARATIVE EXAMPLES 3-4

A 100 ml fermentation spinner flask 1' made by Shibata Haio Company is shown in Figure 3. Lines 10' and 6' were attached to the flask, and it was used as a fermentor.

One hundred milliliters of each of the culture media shown in Table 2 was introduced into the fermentor, and mouse-mouse hybridoma 4C10B6 was seeded at a density of 5×10^4 cells/ml, and cultivated at 37 °C with stirring at 60 rpm. The atmosphere of the space in the fermentor was as shown in Table 2.

In Examples 3 to 7, each of the fluorocarbons indicated in Table 2 was introduced into the fermentor through line 14' so that the concentration of dissolved oxygen in the cultivation liquor became 3 ppm. When the amount of the fluorocarbon residing in the fermentor exceeded a certain limit, it was automatically discharged out of the system through line 6'.

In Comparative Examples 3 and 4, the fluorocarbon was not added.

On the 5th day after the intitiation of cultivation, the density of living cells was measured, and the results are shown in Table 2.

Table 2

		Atmosphere (*)	Fluorocarbon	Average surface area of the fluorocarbon (cm^2/ml)	Culture medium	Density of living cells on the 5th day (cells/ml)	Concentration of antibody (micrograms/ml)
Example	3	N_2	Perfluoro-decalin	20	10% FCS RPMI	9.5×10^5	64
	4	N_2	Fluorinert FC-70	10	10% FCS RPMI	9.8×10^5	68
	5	N_2	Fluorinert FC-72	33	10% FCS RPMI	9.7×10^5	71
	6	N_2	Fluorinert FC-40	18	HB.101	1.1×10^6	72
	7	Air	Fluorinert FC-40	19	10% FCS RPMI	1.1×10^6	69
Comparative example	3	Air	None	-	10% FCS RPMI	1.0×10^6	67
	4	N_2	None	-	10% FCS RPMI	No living cell.	0

(*): All atmospheres contained 5% CO_2.

EXAMPLE 8

A device shown in Figure 4 of the accompanying drawings was used. A glass fermentor 1 having a diameter of 80 mm and a height of 140 mm including a cylindrical rotating filter having a height of 35 mm and an outside diameter of 60 mm (impermeable to cells but permeable to liquid components; G-5 glass fiber in the present example) was sterilized in an autoclave. The fermentor was then charged with 225 ml of RPMI 1640 medium containing 10% fetal bovine serum, and mouse-mouse hybridoma 4C10B6 cells (parent

strain: P3U1) were seeded at a density of 1.5 x 10^5 cells/ml (these cells produce IgG2b antibody). Air containing 5% CO_2 was passed through the upper portion of the fermentor, and the cells were cultivated at 37 °C while rotating the rotating filter at 150 rpm.

On the third day, the cell density was measured. Living cells were found to exist at a density of 9.0 x 10^5 cells/ml, and no dead cells were observed.

On the third day, continuous feeding of a fresh medium of the above composition was started at a rate of 450 ml/day from line 14 in Figure 4. Simultaneously, the cultivation liquor was continuously separated from the cells and taken out of the system through line 13 via filter 15 by means of a pump fitted to the end of the line 13 so that the volume of cultivation liquor in the fermentor became constant at 225 ml. On the 4th day, the concentration of dissolved oxygen in the fermentor dropped below 3 ppm. Therefore, the supplying of air containing 5% CO_2 was stopped, and from line 7, Fluorinert FC-40 (a product of 3M Company) having oxygen dissolved therein was added dropwise from the top of the fermentor so that the amount of dissolved oxygen in it became 3 ppm. The average surface area of the fluorocarbon at this time was 21 cm^2/ml.

A fluorocarbon-withdrawing line 6 is attached to the bottom of the fermentor 1, and a communication line 7 leading to the fermentor is connected to this line so that the position of the interface between the fluorocarbon and the cultivation liquor can be controlled automatically at a fixed position. The position of the interface between the cultivation liquor and the fluorocarbon in the fermentor 1 can be set at a desired level by changing the position of the connecting part between the lines 6 and 7. The fluorocarbon introduced into the cultivation system was automatically supplied to a bubble tower 9 through lines 6 and 8 while its amount residing in the system was maintained constant. Oxygen gas containing 5% CO_2 sterilized through a filter was continuously blown into the bubble tower 9 from its bottom portion through line 10. The fluorocarbon which had oxygen fully dissolved in it in the inside member of the bubble tower 9 resided at the bottom portion of the outside member of the tower. The fluorocarbon was supplied to the cultivation system through line 2 by means of a pump 12 so that the concentration of dissolved oxygen in the cultivation system became 3 ppm.

On the sixth day and thereafter, the rate of feeding the fresh medium from line 14 was changed to 675 ml/day.

The cell densities and the antibody concentrations were measured, and the results are shown in Table 3.

Table 3

Time	Cell density (cells/ml)		Concentration of the antibody (micrograms/ml)
	Living cells	Dead cells	
1st day	1.5 x 10^5	0	0
3rd day	9.0 x 10^5	0	32
4th day	1.9 x 10^6	0	28
6th day	6.5 x 10^6	2.3 x 10^5	64
8th day	1.2 x 10^7	2.1 x 10^5	138
9th day	1.4 x 10^7	3.2 x 10^5	173

EXAMPLE 9

Thirty milliliters of the modified Murashige Skoog medium described in Table 4 below and 30 ml of

Fluorinert FC-40 (a fluorocarbon made by Sumitomo 3M Company) were introduced into a 100 ml Erlenmeyer flask equipped with a dropping port for adding the fluorocarbon dropwise at the upper portion of the flask and a discharge opening for discharging the fluorocarbon at its lower portion and adapted to circulate the fluorocarbon externally. Cultivated cells (green-colored) of Nicotiana tabacum Samson were seeded in an amount of 1.0 g as fresh weight. The air in the upper portion of the flask was replaced by nitrogen, and the flask was stopped. Fluorinert FC-40 having dissolved oxygen in it by aseptic aeration was circulated by means of a peristatic pump by harmonizing introduction into the fermentor with discharging from it. At this time, the introduction of the fluorocarbon was effected by dropping droplets of the fluorocarbon from the dropping port at the upper portion of the flask, and the liquid was discharged from the discharge portion located in the fluorocarbon layer. At the time of dropwise addition, the average surface area of the fluorocarbon was 20 cm^2/ml.

The fermentor was rotated by a rotary shaker at a speed of 120 rpm, and the cultivation was cultivated for 10 days at 25 °C under the illumination of a fluorescence lamp at about 3000 luxes.

All of the cultivated cells were harvested from the fermentor. 9.3 g, as the fresh weight, of green cells were obtained. This shows that the cells had ordinary proliferating ability.

Table 4

Composition of the modified Murashige Skoog medium

Component	Concentration (mg/liter)
Potassium nitrate	1900
Ammonium nitrate	1650
Calcium chloride dihydrate	440
Magnesium sulfate heptahydrate	370
Potassium dihydrogen phosphate	170
[Trace Components]	
Boric acid	6.2
Manganese sulfate tetrahydrate	22.3
Zinc sulfate tetrahydrate	8.6
Potassium iodide	0.83
Sodium molybdate dihydrate	0.25
Copper sulfate pentahydrate	0.025
Cobalt chloride hexahydrate	0.025
[Iron Source]	
Iron (II) sodium ethylenediamine-tetraacetate	38.5
[Vitamins]	
Thiamine hydrochloride	0.40
Inositol	100
Pyridoxine hydrochloride	0.5
Nicotinic acid	0.5
Glysine	2
[Hormones]	
1-Naphthaleneacetic acid	1.86
6-Benzyladenine	0.225
[Carbon source)	
Sucrose	30,000
pH	5.6

EXAMPLE 10

Cultivated cells (green) of Catharanthus roseus were cultivated for 10 days under the same conditions as in Example 1 except that in the culture medium 2,4-dichloroacetic acid was used as the hormone in a

concentration of 0.1 mg/liter.

The grown cells were harvested from the fermentor. Green cells were obtained in an amount of 11.3 g as fresh weight.

The cells were lyophilized and alkaloids were extracted from the cells in a customary manner, and the residue was analyzed by high-performance liquid chromatography. Both qualitatively and quantitatively, hardly any effect by the use of Fluorinert FC-40 was observed.

EXAMPLE 11

Cultivated cells (white) of soybean (Glycine max) were cultivated for 10 days using Fluorinert FC-75 (a fluorocarbon manufactured by Sumitomo 3M Company) (average surface area 23 cm^2/ml) under the same conditions as in Example 1 except that the cultivation was carried out in the dark.

The grown cells were harvested from the fermentor, and 10.5 g, as fresh weight, of white cells were obtained. They showed the same proliferating ability as in an ordinary cultivation.

EXAMPLE 12

In the same way as in Example 9, shoots of Digitalis purpurea were cultivated for 21 days using perfluorodecalin (average surface area 22 cm^2/ml). As a result of the cultivation, 5.3 g, as fresh weight, of shoots were harvested. They showed the same degree of proliferating ability as an ordinary cultivation.

EXAMPLE 13

Three hundred milligrams of Cytodex 1 (Pharmacia Fine Chemicals) (a microcarrier equilibrated with a medium by the method described at page 44 of the Japanese version of Microcarrier Cell Culture Principles & Methods of Pharmacia Fine Chemicals) was introduced into 100 ml of the same culture medium as used in Example 1 charged into the same spinner flask as used in Example 3, and incubated for 1 hour at 37 °C while passing a gaseous mixture composed of 95 % of air and 5 % of CO_2. Thereafter, 5 x 10^5 Vero cells (monkey kidney cells) which had been cultivated in an incubator were seeded. The air in the space in the fermentor was replaced by nitrogen. The cultivation was carried out by supplying oxygen to the cultivation system by Fluorinert FC-40 in the same manner as in Example 3 so that the concentration of dissolved oxygen in the cultivation liquor reached 3 ppm. During this time, the average surface area of the fluorocarbon was 19 cm^2/ml. The cultivation system was sampled properly, and the number of cells was measured by the method described at page 64 of the above-cited technical literature of Pharmacia Fine Chemicals.

Table 5

Time	Cell density (cells/ml)
1st day	6×10^4
2nd day	9×10^5
3rd day	1.1×10^5
5th day	9.0×10^5
7th day	1.1×10^6

EXAMPLE 14

A device shown in Figure 5 was used. A fermentor 1 was made of glass and had an inside diameter of 15 mm and a height of 1,000 mm. It included a sparger 3 (made of SUS 316 stainless steel) for dispersion of a fluorocarbon which had four nozzles with a diameter of 1 mm.

First, 120 ml of the same culture medium as used in Example 1, sterilized by filtration, was charged into the fermentor. Separately, 50 ml of a fluorocarbon (Fluorinert FC-77) which had been deoxygenated with nitrogen gas and sterilized by filtration was introduced into the fermentor. The fluorocarbon was introduced

into line 4, pump 5, line 6, line 8, bubble tower 9, pump 12 and line 13. By operating the pump 5, the fluorocarbon was fed into the cultivation system from the sparger 3. The fluorocarbon which left the sparger was in the form of liquid droplets and had an average surface area of 42 cm^2/ml. A gas composed of 5% of CO_2 and 95 % of air was blown into the bubble tower 9 through line 10. The fluorocarbon which absorbed oxygen was fed into the fermentor automatically through pump 12, line 13, line 4, pump 5 and line 2 so that the concentration of dissolved oxygen in the fermentor became 3 ppm. The fluorocarbon which gathered at the bottom of the fermentor was sent to the bubble tower 9 through lines 6 and 8. Since a communication pipe 7 is attached to the fermentor and the lines 6 and 8, the liquid level of the fluorocarbon in the fermentor was maintained constant.

When the above condition became stable, mouse-human hybridoma H-2 (a human IgG-producing hybridoma obtained by fusing mouse myeloma cells P3-X63-Ag8-U1 with human B cells) was seeded at a cell density of 1.5 x 10^5 cells/ml, and continuously cultivated at 37 °C. The results are shown in Table 6.

Table 6

Time	Cell density (cells/ml)
1st day	1.5 x 10^5
2nd day	2.1 x 10^5
3rd day	4.0 x 10^5
4th day	7.9 x 10^5
5th day	1.2 x 10^6

EXAMPLE 15

A device shown in Figure 6 was used. The same fermentor 1 as used in Example 1 was used. A fluorocarbon dispersing sparger 3 (made of SUS 316 stainless steel) in the fermentor 1 had four holes with a diameter of 2 mm. Each hole was bored so that its distance from the central axis of a rotating filter equalled the arithmetic average of the inside diameter of the fermentor and the outside diameter of the rotating filter 3.

The fermentor was entirely sterilized in an autoclave. The same culture medium as used in Example 1 which had been sterilized by filtration was charged into the fermentor so that the volume of the cultivation liquor became about 225 ml, and mouse-mouse hybridoma 4C10B6 was seeded at a cell density of 1.5 x 10^5 cells/ml. A fluorocarbon (Fluorinert FC-70) sterilized by filtration was charged into the cultivation system in the same manner as in Example 8. The rotating filter was rotated at 30 rpm. By operating pumps 5 and 12, the fluorocarbon was fed into the cultivation system, and a gas composed of 5 % of CO_2 and 95 % of O_2 was continuously blown into bubble tower 9 through line 10. The fluorocarbon which left the sparger 3 formed a liquid column and had an average surface area of 31 cm^2/ml. The cultivation system was maintained at 37 °C, and the cultivation was carried out by automatically operating and stopping the pump 12 so that the concentration of dissolved oxygen in the fermentor was 3 ppm.

On the third day, the density of living cells was 8.1 x 10^5 cells/ml, and no dead cells were observed.

On the third day and thereafter, the same culture medium as in Example 1 was continuously fed at a rate of 450 ml/day from line 14. At the same time, the cultivation liquor was continuously separated from the cells, and taken out of the cultivation system through the rotating filter 15 and line 13 by means of a pump fitted to the end of line 13 so that the volume of the cultivation liquor in the fermentor was maintained constant at 225 ml. On the 6th day and thereafter, the rate of feeding the fresh medium fed from line 14 was changed to 675 ml/day.

The operability of the high-density continuous cultivation by the perfusion method described above was good. The cell densities and the antibody concentrations in the cultivation liquor were measured, and the results are shown in Table 7.

Table 7

Time	Cell density (cells/ml)		Concentration of the antibody (micrograms/ml)
	Living cells	Dead cells	
1st day	1.5×10^5	0	0
3rd day	8.1×10^5	0	29
4th day	1.8×10^6	0	24
6th day	6.3×10^6	0	62
8th day	1.3×10^7	1.6×10^5	141
9th day	1.5×10^7	1.4×10^5	163

EXAMPLE 16

The same cultivation as in Example 15 was carried out except that a sparger 3 having a slit, 1.5 mm wide and 2 cm long, provided at the same position as the nozzle used in Example 15 was used. By using this sparger, a fluorocarbon (Fluorinert FC-70) was formed into a liquid film having a surface area of 19 cm^2/ml.

The operability of the high-density continuous cultivation by the perfusion method described above was good. The cell densities and the antibody concentrations in the cultivation liquor were measured, and the results are shown in Table 8 below.

Table 8

Time	Cell density (cells/ml)		Concentration of the antibody (micrograms/ml)
	Living cells	Dead cells	
1st day	1.5×10^5	0	0
3rd day	9.3×10^5	0	31
4th day	2.1×10^6	0	28
6th day	6.7×10^6	1.0×10^5	59
8th day	1.4×10^7	1.6×10^5	152
9th day	1.4×10^7	2.1×10^5	171

EXAMPLE 17

The same cultivation as in Example 15 was carried out except that Fluorinert FC-77 was used as the fluorocarbon, and the hole diameter of the sparger 3 was changed to 0.1 mm. The average surface area of the fluorocarbon was 270 cm^2/ml.

A very small portion of the cultivation liquor was entrained in the fluorocarbon, but the operability was good.

The cell densities and the antibody concentrations in the cultivation liquor were measured, and the results are shown in Table 9.

Table 9

Time	Cell density (cells/ml)		Concentration of the antibody (micrograms/ml)
	Living cells	Dead cells	
1st day	1.5×10^5	0	0
3rd day	6.1×10^5	0	24
4th day	1.5×10^6	1.2×10^5	19
6th day	5.2×10^6	3.6×10^5	41
8th day	9.0×10^6	8.9×10^5	98
9th day	1.1×10^7	1.3×10^6	124

Claims

1. A method of cultivating animal or plant cells, which consists of contacting a cultivation liquor having animal or plant cells suspended therein with a liquid fluorocarbon having molecular oxygen dissolved therein, characterized in that said cultivation liquor forms a continuous suspension phase which has cell densities of at least 4×10^6 cells/ml, that said contact is made by feeding said liquid fluorocarbon into the cultivation liquor from above the cultivation liquor such that 1 ml of liquid fluorocarbon has a surface area of about 6 to about 300 cm^2 whereby the liquid fluorocarbon falls downward through the cultivation liquor by gravity.

2. The method of claim 1 wherein the liquid fluorocarbon is in the form of a liquid droplet.

3. The method of claim 1 wherein 1 ml of the liquid fluorocarbon has a surface area of about 10 to 200 cm^2.

4. The method of claim 1 wherein the density of the animal cells in the cultivation liquor is at least 6×10^6 cells/ml.

5. The method of claim 1 wherein the cultivation liquor is separated and withdrawn from the cultivation system consisting substantially of the cultivation liquor, the animal or plant cells and the liquid fluorocarbon continuously or stepwise, and in the meantime, a fresh liquid medium in an amount corresponding to the withdrawn cultivation liquor is fed into the cultivation system continuously or stepwise.

6. The method of claim 1 wherein no external positive stirring operation is exerted on the cultivation system consisting substantially of the cultivation liquor and the liquid fluorocarbon.

7. The method of claim 1 wherein the liquid fluorocarbon is substantially nontoxic to the animal or plant cells.

8. The method of claim 1 wherein the liquid fluorocarbon is a perfluorocarbon.

9. The method of claim 1 wherein the liquid fluorocarbon is selected from the group consisting of perfluoroalkanes having 6 to 20 carbon atoms, perfluorocycloalkanes having 5 to 14 carbon atoms which may be substituted by perfluoroalkyl groups having 1 to 5 carbon atoms, perfluorofurans substituted by perfluoroalkyl groups having 1 to 7 carbon atoms, perfluorotetrahydrofurans substituted by perfluoroalkyl groups having 1 to 7 carbon atoms, perfluoropyrans substituted by perfluoroalkyl groups having 1 to 6 carbon atoms, perfluorotetrahydropyrans substituted by perfluoroalkyl groups having 1 to 6 carbon atoms, perfluoroadamantanes which may be substituted by perfluoroalkyl groups having 1 to 5 carbon atoms, and tertiary amino group substituted products of said fluorocarbons.

10. The method of claim 1 wherein the animal cells are anchorage-independent cells, and are suspended in the cultivation liquor without being supported on a carrier.

11. The method of claim 1 wherein the animal cells are anchorage-independent, and are suspended in the cultivation liquor while being immobilized with a gel.

12. The method of claim 1 wherein the animal cells are anchorage-dependent cells, and are suspended in the cultivation liquor while being supported on a carrier.

13. The method of claim 1 wherein the cells are animal cells.

14. The method of claim 1 wherein the cultivation liquor having the animal cells suspended therein is a serum-free culture medium.

15. A method for cultivating animal or plant cells, which comprises
(1) feeding a liquid fluorocarbon having molecular oxygen dissolved therein from above into a cultivation tank containing a continuous phase of a cultivation liquor having animal or plant cells suspended therein in a density of at least 4×10^5 cells/ml to thereby contact them with each other while 1 ml of the liquid fluorocarbon has a surface area of about 6 to about 300 cm^2;
(2) withdrawing a heavy phase composed substantially of the fluorocarbon from the bottom of the cultivation tank;
(3) dissolving molecular oxygen in the fluorocarbon in the heavy phase; and
(4) thereafter using the liquid fluorocarbon having molecular oxygen dissolved therein in step (1).

16. The method of claim 15 wherein the cultivation liquor is separated and withdrawn from the cultivation system consisting substantially of the cultivation liquor, the animal or plant cells and the liquid fluorocarbon continuously or stepwise, and in the meantime, a fresh liquid medium in an amount corresponding to the withdrawn cultivation liquor is fed into the cultivation system continuously or stepwise.

Revendications

1. Procédé de culture de cellules animales ou végétales, qui consiste à mettre en contact une liqueur de culture contenant des cellules animales ou végétales en suspension avec un fluorocarbure liquide contenant de l'oxygène moléculaire dissous, caractérisé en ce que ladite liqueur de culture forme une phase continue de suspension qui présente des densités cellulaires d'au moins 4×10^6 cellules/ml, en ce que ladite mise en contact est effectuée en introduisant ledit fluorocarbure liquide dans la liqueur de culture par le dessus de la liqueur de culture de telle manière que 1 ml de fluorocarbure liquide offre une surface de contact d'environ 6 à environ 300 cm^2, en sorte que le fluorocarbure liquide tombe par gravité à travers la liqueur de culture.

2. Procédé selon la revendication 1, dans lequel le fluorocarbure liquide est sous la forme de gouttelette liquide.

3. Procédé selon la revendication 1, dans lequel 1 ml du fluorocarbure liquide offre une surface de contact d'environ 10 à 200 cm^2.

4. Procédé selon la revendication 1, dans lequel la densité des cellules animales dans la liqueur de culture est d'au moins 6×10^6 cellules/ml.

5. Procédé selon la revendication 1, dans lequel la liqueur de culture est séparée et retirée en continu ou par étapes du système de culture constitué essentiellement de la liqueur de culture, des cellules animales ou végétales et du fluorocarbure liquide, et, pendant ce temps, un milieu liquide frais est introduit en continu ou par étapes dans le système de culture en une quantité correspondant à la liqueur de culture retirée.

6. Procédé selon la revendication 1, dans lequel aucune opération effective d'agitation extérieure n'est exercée sur le système de culture constitué essentiellement de la liqueur de culture et du fluorocarbure liquide.

7. Procédé selon la revendication 1, dans lequel le fluorocarbure liquide est sensiblement atoxique pour les cellules animales ou végétales.

8. Procédé selon la revendication 1, dans lequel le fluorocarbure liquide est un perfluorocarbure.

9. Procédé selon la revendication 1, dans lequel le fluorocarbure liquide est choisi dans la classe formée par les perfluoroalcanes ayant 6 à 20 atomes de carbone, les perfluorocycloalcanes ayant 5 à 14 atomes de carbone qui peuvent être substitués par des groupes perfluoroalkyle ayant 1 à 5 atomes de carbone, les dérivés de substitution du perfluorofuranne par des groupes perfluoroalkyles ayant 1 à 7 atomes de carbone, les dérivés de substitution du perfluorotétrahydrofuranne par des groupes perfluoroalkyle ayant 1 à 7 atomes de carbone, les dérivés de substitution du perfluoropyranne par des groupes perfluoroalkyle ayant 1 à 6 atomes de carbone, les dérivés de substitution du perfluorotétrahydropyranne par des groupes perfluoroalkyle ayant 1 à 6 atomes de carbone, le perfluoroadamantane et ses dérivés de substitution par des groupes perfluoroalkyle ayant 1 à 5 atomes de carbone, et les produits de substitution desdits fluorocarbures par des groupes amino tertiaires.

10. Procédé selon la revendication 1, dans lequel les cellules animales sont des cellules ne nécessitant pas de fixation, et sont mises en suspension dans la liqueur de culture sans être fixées à un support.

11. Procédé selon la revendication 1, dans lequel les cellules animales ne nécessitent pas de fixation et sont mises en suspension dans la liqueur de culture en étant immobilisées par un gel.

12. Procédé selon la revendication 1, dans lequel les cellules animales sont des cellules nécessitant une fixation et sont mises en suspension dans la liqueur de culture en étant fixées à un support.

13. Procédé selon la revendication 1, dans lequel les cellules sont des cellules animales.

14. Procédé selon la revendication 1, dans lequel la liqueur de culture contenant les cellules animales en suspension est un milieu de culture sans sérum.

15. Procédé pour cultiver des cellules animales ou végétales, qui consiste à

(1) introduire par le dessus un fluorocarbure liquide contenant de l'oxygène moléculaire dissous dans une cuve de culture contenant une phase continue d'une liqueur de culture contenant des cellules animales ou végétales en suspension en une densité d'au moins 4×10^6 cellules/ml, pour les amener ainsi mutuellement en contact en assurant que 1 ml du fluorocarbure liquide offre une surface de contact d'environ 6 à environ 300 cm^2 ;

(2) retirer une phase lourde constituée essentiellement du fluorocarbure par le fond de la cuve de culture ;

(3) dissoudre de l'oxygène moléculaire dans le fluorocarbure de la phase lourde ; et

(4) utiliser ensuite dans l'étape (1) le fluorocarbure liquide contenant de l'oxygène moléculaire dissous.

16. Procédé selon la revendication 15, dans lequel la liqueur de culture est séparée et retirée en continu ou par étapes du système de culture constitué essentiellement de la liqueur de culture, des cellules animales ou végétales et du fluorocarbure liquide, et, pendant ce temps, un milieu liquide frais est introduit en continu ou par étapes dans le système de culture en une quantité correspondant à la liqueur de culture retirée.

Patentansprüche

1. Verfahren zur Züchtung tierischer oder pflanzlicher Zellen durch Behandlung einer Kultivationsflüssigkeit, in der tierische oder pflanzliche Zellen suspendiert sind, mit einem flüssigen Fluorkohlenwasserstoff, in dem molekularer Sauerstoff gelöst ist, dadurch **gekennzeichnet,** daß die Züchtungsflüssigkeit eine kontinuierliche Suspensionsphase bildet, die Zelldichten von mindestens 4×10^6 Zellen/ml aufweist, daß die Behandlung erfolgt, indem der flüssige Fluorkohlenwasserstoff in die Züchtungsflüssigkeit über der Züchtungsflüssigkeit eingeleitet wird, so daß 1 ml flüssiger Fluorkohlenwasserstoff eine Oberfläche von etwa 6 bis etwa 300 cm^2 aufweist, wodurch der flüssige Fluorkohlenwasserstoff abwärts durch die Züchtungsflüssigkeit bedingt durch die Schwerkraft fällt.

2. Verfahren nach Anspruch 1, dadurch **gekennzeichnet,** daß der flüssige Fluorkohlenwasserstoff in Form von Flüssigkeitströpfchen vorliegt.

3. Verfahren nach Anspruch 1, dadurch **gekennzeichnet,** daß 1 ml flüssiger Fluorkohlenwasserstoff eine Oberfläche von etwa 10 bis 200 cm^2 besitzt.

4. Verfahren nach Anspruch 1, dadurch **gekennzeichnet,** daß die Dichte der tierischen Zellen in der Züchtungsflüssigkeit mindestens 6×10^6 Zellen/ml beträgt.

5. Verfahren nach Anspruch 1, dadurch **gekennzeichnet,** daß die Züchtungsflüssigkeit aus dem Züchtungssystem, das im wesentlichen aus der Züchtungsflüssigkeit, den tierischen oder pflanzlichen Zellen und dem flüssigen Fluorkohlenwasserstoff besteht, kontinuierlich oder stufenweise abgetrennt und entnommen wird, und daß zwischenzeitlich frisches flüssiges Medium in einer Menge, die der entnommenen Züchtungsflüssigkeit entspricht, in das Züchtungssystem kontinuierlich oder stufenweise eingeleitet wird.

6. Verfahren nach Anspruch 1, dadurch **gekennzeichnet,** daß auf das Züchtungssystem, das im wesentlichen aus der Züchtungsflüssigkeit und dem flüssigen Fluorkohlenwasserstoff besteht, kein äußerer positiver Rührvorgang ausgeübt wird.

7. Verfahren nach Anspruch 1, dadurch **gekennzeichnet,** daß der flüssige Fluorkohlenwasserstoff im wesentlichen gegenüber den tierischen oder pflanzlichen Zellen nichttoxisch ist.

8. Verfahren nach Anspruch 1, dadurch **gekennzeichnet,** daß der flüssige Fluorkohlenwasserstoff ein Perfluorkohlenwasserstoff ist.

9. Verfahren nach Anspruch 1, dadurch **gekennzeichnet,** daß der flüssige Fluorkohlenwasserstoff ausgewählt wird aus der Gruppe, die besteht aus Perfluoralkanen mit 6 bis 20 Kohlenstoffatomen, Perfluorcycloalkanen mit 5 bis 14 Kohlenstoffatomen, die durch Perfluoralkylgruppen mit 1 bis 5 Kohlenstoffatomen substituiert sein können, Perfluorfuranen, die durch Perfluoralkylgruppen mit 1 bis 7 Kohlenstoffatomen substituiert sein können, Perfluortetrahydrofuranen, die durch Perfluoralkylgruppen mit 1 bis 7 Kohlenstoffatomen substituiert sein können, Perfluorpyranen, die durch Perfluoralkylgruppen mit 1 bis 6 Kohlenstoffatomen substituiert sein können, Perfluortetrahydropyranen, die durch Perfluoralkylgruppen mit 1 bis 6 Kohlenstoffatomen substituiert sein können, Perfluoradamantanen, die durch Perfluoralkylgruppen mit 1 bis 5 Kohlenstoffatomen substituiert sein können, und mit einer tertiären Aminogruppe substituierten Produkten der genannten Fluorkohlenwasserstoffe.

10. Verfahren nach Anspruch 1, dadurch **gekennzeichnet,** daß die tierischen Zellen verankerungsunab-

hängige Zellen sind und in der Züchtungsflüssigkeit ohne, daß sie an einen Träger gebunden sind, suspendiert sind.

11. Verfahren nach Anspruch 1, dadurch **gekennzeichnet**, daß die tierischen Zellen verankerungsunabhängig sind und in der Züchtungsflüssigkeit suspendiert sind, während sie mit einem Gel immobilisiert sind.

12. Verfahren nach Anspruch 1, dadurch **gekennzeichnet**, daß die tierischen Zellen verankerungsabhängige Zellen sind und in der Züchtungsflüssigkeit suspendiert sind, während sie an einen Träger gebunden sind.

13. Verfahren nach Anspruch 1, dadurch **gekennzeichnet**, daß die Zellen tierische Zellen sind.

14. Verfahren nach Anspruch 1, dadurch **gekennzeichnet**, daß die Züchtungsflüssigkeit, in der die tierischen Zellen suspendiert sind, ein serumfreies Kulturmedium ist.

15. Verfahren zur Züchtung von tierischen oder pflanzlichen Zellen, dadurch **gekennzeichnet**, daß
(1) ein flüssiger Fluorkohlenwasserstoff, der molekularen Sauerstoff darin enthält, von oberhalb in einen Züchtungstank geleitet wird, der eine kontinuierliche Phase der Züchtungsflüssigkeit enthält, in der tierische oder pflanzliche Zellen in einer Dichte von mindestens 4×10^6 Zellen/ml suspendiert sind, und daß diese miteinander in Berührung kommen, während 1 ml des flüssigen Fluorkohlenwasserstoffs eine Oberfläche von etwa 6 bis etwa 300 cm^2 besitzt;
(2) die schwere Phase, die im wesentlichen aus dem Fluorkohlenwasserstoff besteht, aus dem Boden des Züchtungstanks entnommen wird;
(3) molekularer Sauerstoff in dem Fluorkohlenwasserstoff in der schweren Phase aufgelöst wird; und
(4) anschließend der flüssige Fluorkohlenwasserstoff, in dem der molekulare Sauerstoff gelöst ist, bei der Stufe (1) verwendet wird.

16. Verfahren nach Anspruch 15, dadurch **gekennzeichnet**, daß die Züchtungsflüssigkeit aus dem Züchtungssystem, das im wesentlichen aus der Züchtungsflüssigkeit, den tierischen oder pflanzlichen Zellen und dem flüssigen Fluorkohlenwasserstoff besteht, kontinuierlich oder stufenweise abgetrennt und entnommen wird, und daß zwischenzeitlich ein frisches flüssiges Medium in einer Menge, die der entnommenen Züchtungsflüssigkeit entspricht, kontinuierlich oder stufenweise in das Züchtungssystem geleitet wird.

FIG. 1

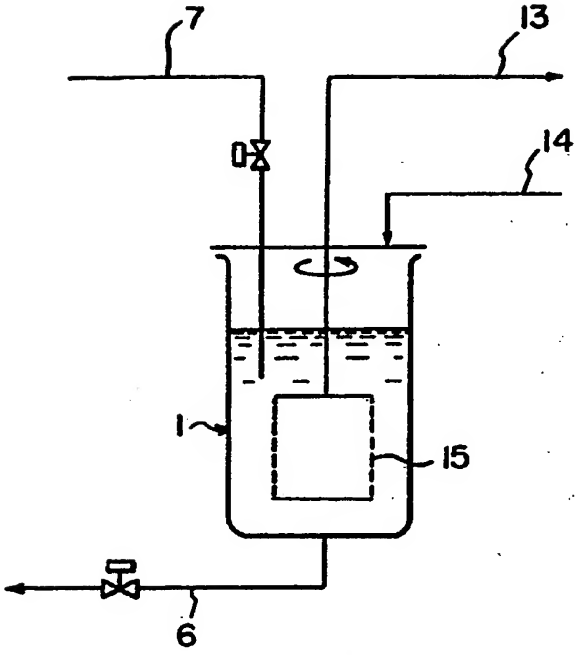

FIG. 2

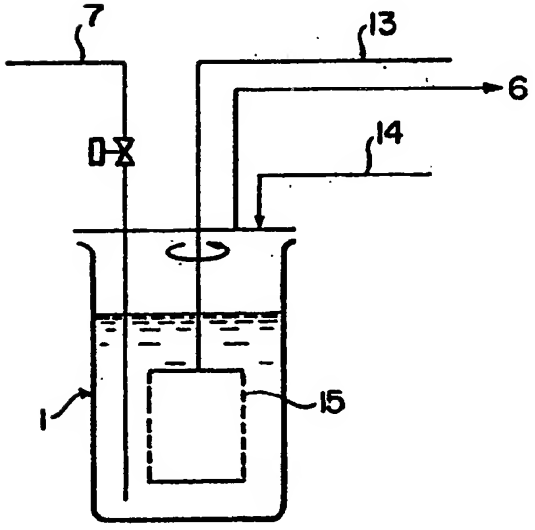

FIG. 3

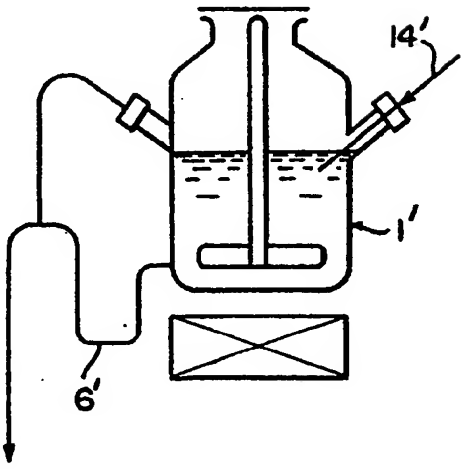

FIG. 4

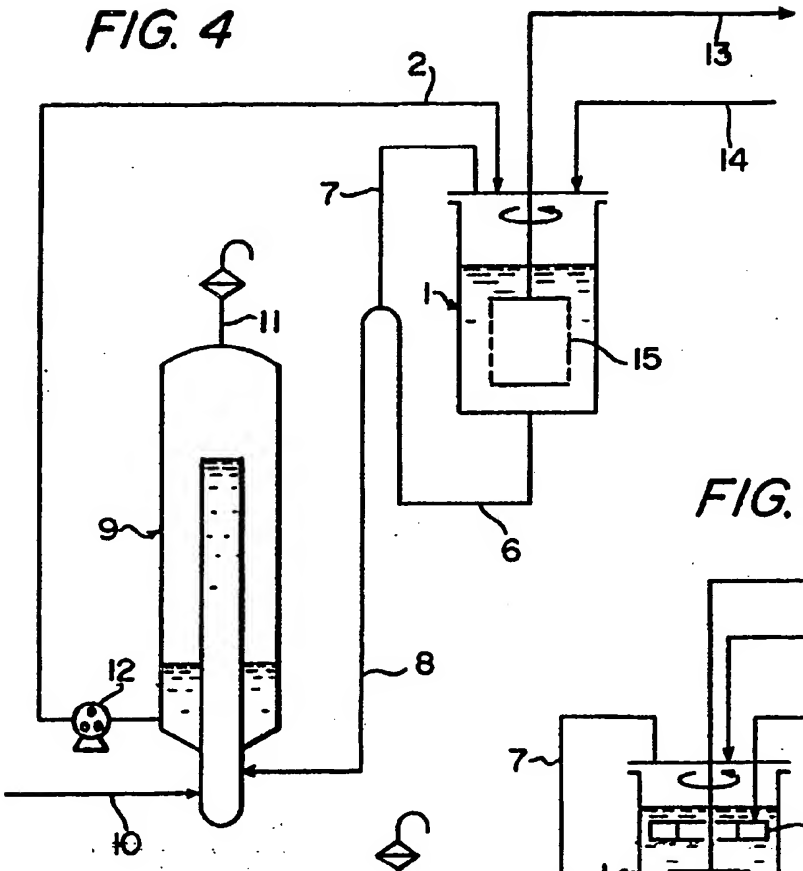

FIG. 6

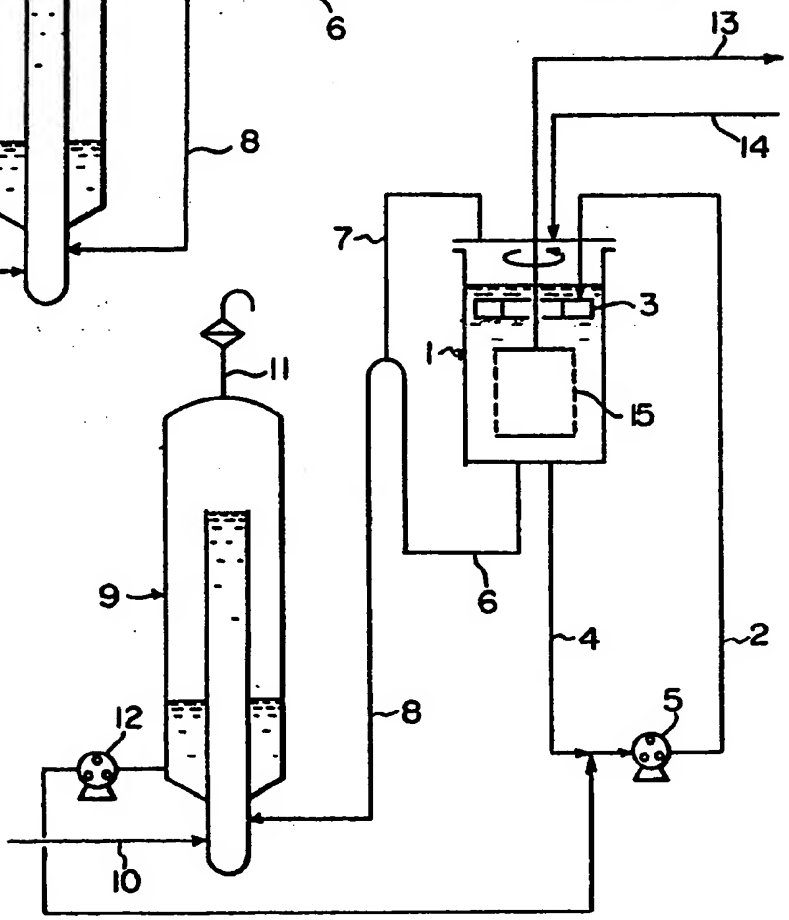

FIG. 5